RESEARCH NOTE

The $hsr\omega^{05241}$ allele of the noncoding $hsr\omega$ gene of *Drosophila melanogaster* is not responsible for male sterility as reported earlier

AKANKSHA, MOUSHAMI MALLIK, ROSHAN FATIMA and S. C. LAKHOTIA*

Cytogenetics Laboratory, Department of Zoology, Banaras Hindu University, Varanasi 221 005, India

An earlier report from our laboratory (Rajendra *et al*. 2001) published in this journal claimed that insertion of the P-transposon at −130 bp position in the *hsrω* promoter was responsible for excessive clustering of the omega speckles in cyst cells in testes, which in turn caused the sterility of $hsr\omega^{05241}$ homozygous males. The present communication is to report that the above conclusion was erroneous since the male sterility of the $hsr\omega^{05241}$ homozygous males is actually due to a 2nd site mutation ms^{21}, which appears to be a novel gene in the 61B1 region of chromosome 3. Further, the ms^{21} mutation, but not the P-transposon insertion in the *hsrω* gene promoter, is associated with clustering of omega speckles in cyst cells.

The *hsrω* gene is developmentally expressed in almost all the cell types from blastoderm to adult stage, it is inducible by environmental changes and produces multiple noncoding transcripts (Lakhotia 2003). The large nucleus, limited hsrω-n transcript (>10 kb), organizes the nucleoplasmic omega speckles with which a variety of RNA processing proteins, especially the hnRNPs, remain associated. These fine speckles coalesce together to form large aggregates in response to various stress conditions, and resume the speckled nucleoplasmic distribution upon recovery from stress. The omega speckles and their aggregates are suggested to act as dynamic sink to regulate the availability of hnRNPs and related proteins in the nucleus (Lakhotia *et al*. 1999; Prasanth *et al*. 2000). The *l(3)05241*, a *P-LacZ-rosy*$^+$ transposon insertion allele of the *hsrω* gene of *Drosophila melanogaster*, was generated as part of the P-transposon insertion induced gene-disruption programme and was originally reported as a recessive lethal allele of the *hsrω* gene (Spradling *et al*. 1995). Subsequently, the mutation responsible for recessive lethality was found to be separable from the P-transposon insertion in the *hsrω* gene. Lakhotia *et al*. (2001), mapped the *P-LacZ-rosy*$^+$ transposon insertion to −130 bp position of the *hsrω* promoter and named this homozygous viable allele as $hsr\omega^{05241}$. An earlier paper from our laboratory (Rajendra *et al*. 2001), reported that the $hsr\omega^{05241}$ allele is a recessive gain of function mutation responsible for clustering of the omega speckles in cyst cells of unstressed testis of $hsr\omega^{05241}$ homozygous males and for their sterility. However, subsequent studies in our laboratory showed that the conclusion of Rajendra *et al*. (2001) that the P-transposon insertion in $hsr\omega^{05241}$ allele is responsible for recessive male sterility is erroneous. The present communication is to rectify the earlier conclusion and to report that the male sterility in the original $hsr\omega^{05241}$ homozygous males is actually due to a 2nd site mutation that we have now mapped to 61B1 region of chromosome 3.

During the course of his doctoral studies on the $hsr\omega^{05241}$ allele in the laboratory, Rajendra (2000) generated a series of excision lines through mobilization of the P-transposon, using the Δ2-3 P-transposase source (Cooley *et al*. 1988). Several of these excision lines, with the P-transposon excised out of the *hsrω* promoter, showed recessive late larval/early pupal lethality. These lines were initially named as pl^2, pl^4, pl^9, pl^{10} *(pupal lethal)* etc. To verify if their recessive lethal phenotype was due to any mutation in the *hsrω* gene, the *pl* alleles were brought in trans with $hsr\omega^{05241}$ or with the deficiencies, *Df(3R)GC14* and $Df(3R)e^{Gp4}$, that uncover the *hsrω* gene. The lethal phenotype of all the *pl* lines was complemented by $hsr\omega^{05241}$ as well as the two deficiencies. However, all $pl/hsr\omega^{05241}$ males were sterile while $pl/Df(3R)GC14$ or $pl/Df(3R)e^{Gp4}$ males were fertile. We also sequenced the promoter region of the *hsrω* gene in the *pl* mutant allele carrying chromosomes. Surprisingly, the base sequences of the *hsrω* promoter in all the *pl* chromo-

*For correspondence. E-mail: lakhotia@bhu.ac.in.

Keywords. *hsr omega*; noncoding RNA; cyst cells; omega speckles.

somes were generally comparable to that of the wild type (not shown), except for a few nonspecific base changes. Subsequently, the mutations responsible for lethality and male sterility in *pl* lines were found to be separable and the larval/pupal lethal mutation was mapped to the 94E13–94F1 region of chromosome 3 (Sengupta 2005).

Taken together these results questioned the conclusion of Rajendra *et al*. (2001) that male sterility in $hsr\omega^{05241}$ was because of the P-transposon insertion in the *hsrω* gene promoter and raised the possibility that, mutation at some other site in this chromosome was responsible for the recessive male sterility. Therefore, we initiated a series of fresh crosses (see figure 1) to allow free recombination in the $hsr\omega^{05241}$ chromosome by crossing the $hsr\omega^{05241}/TM6B$ flies with ry^{-}/ry^{-} flies. The progeny $hsr\omega^{05241}/ry^{-}$ females were crossed with *TM3/TM6B* males (for details of the various mutant genes and balancer chromosomes, see Lindslay and Zimm 1991). The progeny flies carrying the *TM3* balancer chromosome (with dominant stubble bristles and recessive rosy-eye markers) were either red eyed, due to the presence of P-$LacZ{-}rosy^{+}$ transposon ($hsr\omega^{05241P*}/TM3$) or rosy eyed, signifying the absence of the P-transposon ($ry^{-*}/TM3$). The $hsr\omega^{05241P*}$ and ry^{-*} refers to putative recombinant chromosomes. Several hundreds of each of these two types of flies were pair-mated with the parental $hsr\omega^{05241}/TM6B$ flies (figure 1) and the fertility status of $hsr\omega^{05241P*}/hsr\omega^{05241}$ and $ry^{-*}/hsr\omega^{05241}$ progeny males was checked. Contrary to the results expected on the claim of Rajendra *et al*. (2001), it was found that about 40–45% of the F_3 $hsr\omega^{05241P*}/hsr\omega^{05241}$ males fertile and likewise, about 40–45% F_3 $ry^{-*}/hsr\omega^{05241}$ males were sterile. It was clear, therefore, that the P-transposon insertion in the *hsrω* promoter and mutation for male sterility can recombine with a high frequency.

PCR amplification using appropriate primers (Lakhotia *et al*. 2001) and subsequent sequencing of the amplicon of the *hsrω* promoter region of the rosy-eyed male sterile ry^{-*}/ry^{-*} flies further confirmed that the recombinant chromosome indeed did not carry the P-transposon insertion or any other lesion at or around the −130 region of the *hsrω* promoter (not shown). Thus we conclude that the recessive male sterility, earlier claimed by Rajendra *et al*. (2001) to be due to the P-transposon insertion at the −130 position of *hsrω* promoter of the $hsr\omega^{05241}$ allele, is actually due to the second site mutation on chromosome 3. In order to avoid ambiguity, the $hsr\omega^{05241}$ allele in the homozygous male fertile stock will, now onward be referred to as $hsr\omega^{05241P*}$.

Using a series of deletions distributed along the length of chromosome 3, the recessive mutation (named as ms^{21}) responsible for male sterility has been mapped to the 61B1 region on the tip of left arm of chromosome 3; the ms^{21} homozygous males are completely sterile and show exactly the same kind of abnormalities during spermatogenesis as reported by Rajendra *et al*. (2001) for the $hsr\omega^{05241}$ homozygous male flies (R. Fatima and S. C. Lakhotia, unpublished data). That the ms^{21} mutation was also present in the original $hsr\omega^{05241}$ chromosome and was responsible for the male sterile phenotype, was confirmed by checking the fertility of $ms^{21}/hsr\omega^{05241}$ male flies — all such males were found to be sterile like the $hsr\omega^{05241}$ homozygous males. The ms^{21} mutation is being further characterized.

Rajendra *et al*. (2001) also reported clustering of omega speckles in cyst cells of unstressed $hsr\omega^{05241}$ homozygous testes, comparable to those seen in other cells following heat shock (Prasanth *et al*. 2000). It is interesting that the $hsr\omega^{05241P*}$ homozygous fertile males do not show any significant clustering of omega speckles in cyst cells while the cyst cells in testes of the original $hsr\omega^{05241}$ and the ms^{21} homozygous sterile males show clustering of omega speckles (see figure 2). Thus, it appears that the clustering of omega speckles does not result from the P-transposon insertion in the *hsrω* promoter *per se* but seems to be associated with the ms^{21} mutation. In this respect, the earlier conclusion

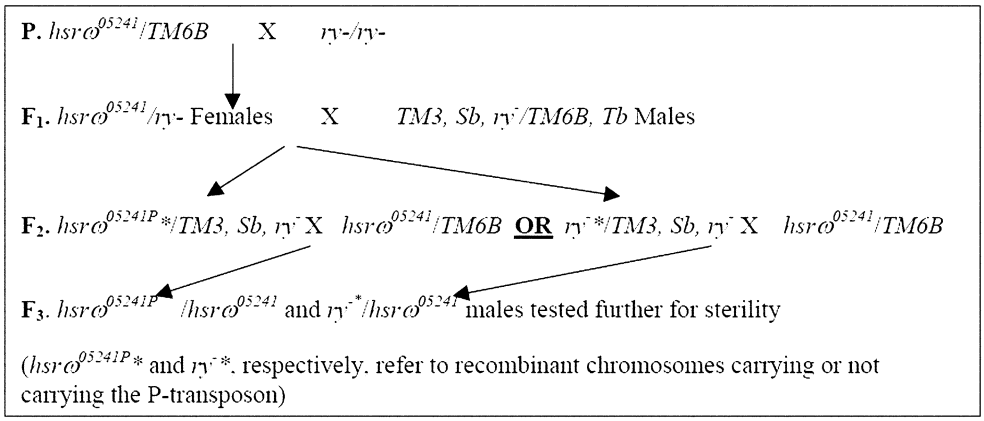

Figure 1. Scheme of crosses to check if the P-transposon insertion in the $hsr\omega^{05241}$ allele and the recessive male sterility can be separated by recombination.

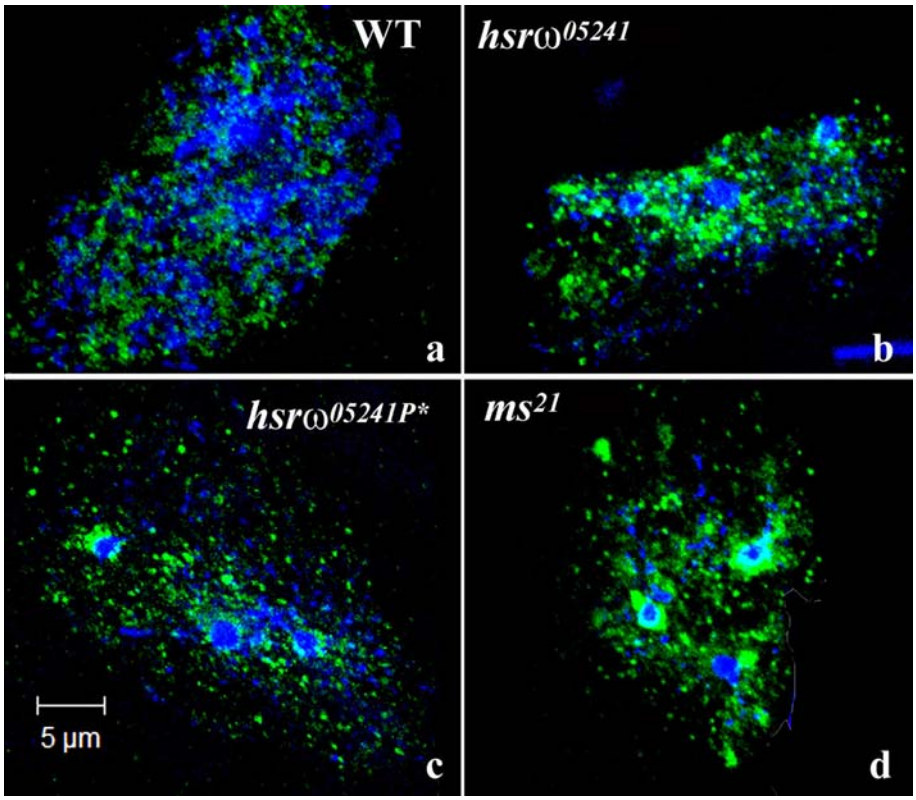

Figure 2. Confocal images (projections) of omega speckles, visualized by immunofluorescence staining with an anti-HRB87F antibody (P11), in cyst cells of: *a*, wild type; *b*, *hsrω^{05241}*; *c*, *hsrω^{05241*}* and *d*, *ms^{21}* males. Note the fine nucleoplasmic speckles in *a* and *c* but larger omega speckles in *b* and *d*. Immunostaining with the P11 antibody (green) and DAPI staining for DNA (blue) was carried out as described earlier (Prasanth *et al*. 2000). Scale bar (5μm) is same for all the panels.

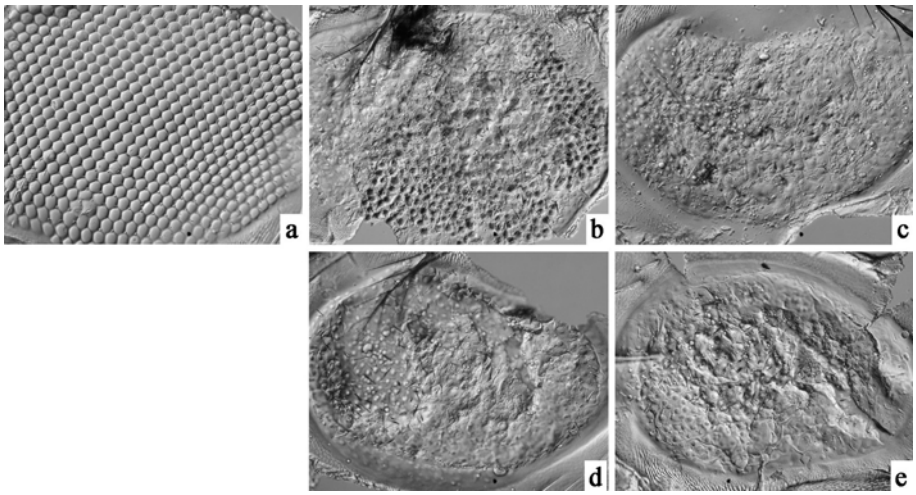

Figure 3. Nail-polish imprints (Arya and Lakhotia 2006) of adult eyes of: *a*, wild type; *b*, *GMR-Gal4/UAS-127Q*; +/+; *c*, *GMR-Gal4/UAS-127Q*; *hsrω^{05241}*/+; *d*, *GMR-Gal4/UAS-127Q*; *hsrω^{05241*}*/+, and *e*, *GMR-Gal4/UAS-127Q*; *ms^{21}*/+ male flies. Note the significant enhancement in polyQ induced neurodegeneration in *c, d* and *e* compared to that in *b*.

(Rajendra *et al*. 2001) that clustering of omega speckles in cyst cells may be causally related to male sterility may still be valid.

The $hsr\omega^{05241}$ allele has also been reported to enhance the neuro-toxicity in fly models of polyQ disorders (Fernandez-Funez *et al*. 2000; Sengupta and Lakhotia 2006). In view of the above results, we also tested if the enhancement of neuro-toxicity in expanded polyQ expressing eye discs by the $hsr\omega^{05241}$ mutant background was due to the P-transposon insertion in the *hsrω* promoter or due to the ms^{21} mutation. Our observations on the eye phenotype in flies expressing polyQ in developing eye discs (Sengupta and Lakhotia 2006) reveal (see figure 3) that, single copy of the P-transposon insertion ($hsr\omega^{05241P^*}$) as well as the ms^{21} mutation enhances the polyQ-induced neurodegeneration like the original $hsr\omega^{05241}$ chromosome, more so in males (M. Mallik and Lakhotia, unpublished data).

An obvious question is why the 2nd site mutation responsible for recessive male sterility in the original $hsr\omega^{05241}$ chromosome was not identified by Rajendra *et al*. (2001), especially when these authors claimed to have undertaken an exercise to recombine out any other mutation in this stock. It is unlikely that the different results obtained now and in 2001 stem from a genetic change in the stock during the interval. It is more likely to be due to an error in either the crosses for free recombination that were made in the 2001 study or to an error in interpretation of the results of the crosses. In either case, S. C. Lakhotia, as the communicating author in the Rajendra *et al*'s 2001 paper and in the present communication, regrets the erroneous conclusion in the earlier paper that the recessive male sterility of $hsr\omega^{05241}$ homozygotes was due to the P-transposon insertion in the *hsrω* promoter. As reported here, the male sterility of the $hsr\omega^{05241}$ homozygous males is actually due to a 2nd site mutation ms^{21}, a novel gene in the 61B1 region of chromosome 3. Further, the ms^{21} mutation, but not the P-transposon insertion in the *hsrω* gene promoter, is associated with clustering of omega speckles in cyst cells.

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Received 5 September 2007; accepted 10 September 2007
Published on the Web: 29 February 2008